

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q 1/70, G01N 33/53, C12N 7/00, 7/02, 5/00	A1	(11) International Publication Number: WO 97/24461 (43) International Publication Date: 10 July 1997 (10.07.97)
(21) International Application Number: PCT/US96/20827 (22) International Filing Date: 27 December 1996 (27.12.96) (30) Priority Data: 08/578,189 29 December 1995 (29.12.95) US (71) Applicant (for all designated States except US): UNIVERSITY HOSPITALS OF CLEVELAND [US/US]; 11100 Euclid Avenue, Cleveland, OH 44106 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): HUANG, Yung, T. [-/US]; 4890 Lindsey Lane, Richmond Heights, OH 44143 (US). (74) Agent: TRITT, William, C.; Renner, Otto, Boisselle & Sklar, 19th floor, 1621 Euclid Avenue, Cleveland, OH 44115 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHODS OF CULTURING AND ASSAYING A VIRUS (57) Abstract This invention relates to a method for culturing a virus including the steps of: (A) providing cells from a cell line susceptible to infection by the virus and a specimen; (B) treating the cells with a compound of formula RC(O)Q, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; (C) inoculating the treated cells with the specimen; and (D) incubating the inoculated cells to allow viral growth to proceed.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

METHODS OF CULTURING AND ASSAYING A VIRUS

5

Technical Field of the Invention

This invention generally relates to the field of diagnostic virology. More particularly, the invention relates to methods for culturing and assaying a virus and diagnostic kits and cell lines for doing the same.

Background of the Invention

10

Viruses and other microorganisms which infect humans, as well as other organisms, are numerous. Many of these viruses are associated with assorted clinical manifestations. Early detection of the viral etiological agents causing the clinical manifestation is desirable for effective treatment and management of the infected organism. Detection of even small concentrations of virus is desirable.

15

Tissue culture techniques using various cell lines are the most widely used means of assaying viruses. Numerous cell lines have been developed for culturing viruses. However, relatively few are useful because of the tendency of many cell lines to be unstable, to develop undesirable characteristics, or to be insensitive for viral growth. Accord-

20

-2-

ingly, a more sensitive cell line for isolation and detection of viruses is a constant goal of diagnostic virologists.

Ideal cells for culturing viruses are cells which are sensitive for a variety of viral pathogens. However, such cell cultures have yet to be developed. Thus, several different cell cultures are required to diagnose various virus types. Such cell cultures are either primary cells or cell strains or lines arising from primary cells. Primary cells are started from cells taken directly from the tissue or organs of an organism. Primary cells are considered to be primary cells until subcultured for the first time wherein they are regarded as cell lines. Primary cells are expended by use so that a continuous supply of primary cells from new tissue from living donors is needed. This may require sacrifice of the donor. Furthermore, each new batch of primary cells poses a risk of endogenous viral, bacterial or fungal contamination.

Use of cell lines may minimize these problems. Cell lines arise from the first subculturing of primary cells. Such cell lines may be finite, i.e., are able to be subcultured only a limited number of times before senescence, or continuous, i.e., are able to be subcultured an infinite number of times. Such continuous cell lines are referred to as "established" cell lines when it is demonstrated the cell line may be subcultured indefinitely in vitro.

Recently, an improved shell vial technique has been developed for the detection of viruses from clinical specimens. This method may be combined with blind immunohistochemistry staining of the inoculated culture with viral specific antibody to identify the virus. This technique is commonly used in diagnostic virology laboratories. However, the method relies on the susceptibility of the cell line, used for virus culture,

-3-

to viral infection. Low susceptibility of the cell line to viral infection results in poor diagnostic sensitivity.

Another recent advancement, polymerase chain reaction (PCR) technology, has been used to detect slow growing viruses, viruses which cannot be grown in cell culture, or viruses which are difficult to recover in cell culture from a specific specimen site. An example is herpes simplex virus from cerebrospinal fluid specimens. However, this technique detects viral nucleic acid and not infectious virus. When it is possible, the detection of infectious virus is often preferred because it correlates more with clinical manifestation. Accordingly, PCR detection of viral nucleic acid may only be indicative of the presence of remnants of a past infection or the presence of a latent infection.

The effect of butyrate on mammalian cells has been discussed. Prasad, K.N. et al, (1976) "*Effect of Sodium Butyrate on Mammalian Cells in Culture: a Review*," *In Vitro* 12, 125-132. The effect of sodium butyrate on various specific cell-virus interactions has also been examined. For instance between: (1) Epstein-Barr Virus/human lymphoblastoid cells, Luka, Janos et al, (1979) "*Induction of the Epstein-Barr Virus (EBV) Cycle in Latently Infected Cells by n-Butyrate*," *Virology*, 94, 228-231; (2) herpes simplex virus/neuroblastoma cells, Ash, Ronald J., (1986) "*Butyrate Induced Reversal of Herpes Simplex Virus Restriction In Neuroblastoma Cells*," *Virology*, 155, 584-592; (3) human endothelial cells/cytomegalovirus, Radsak, K., et al, (1989) "*Induction by Sodium Butyrate of Cytomegalovirus Replication in Human Endothelial Cells*," *Arch. Virology*, 107, 151-158; and (4) human epithelial cells/cytomegalovirus, Tanaka, J. et al, (1991) "*Sodium Butyrate-Inducible Replication*

-4-

of Human cytomegalovirus in a Human Epithelial Cell Line," Virology, 185, 271-280.

International patent publication WO 89/06686 discloses use of butyric acid and salts thereof to enhance the efficiency of specific protein production by cultured eukaryotic cells.

Summary of the Invention

This invention relates to a method of culturing a virus including the steps of: (A) providing cells from a cell line susceptible to infection by the virus and a specimen; (B) treating the cells with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX, or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; (C) inoculating the treated cells with the specimen; and (D) incubating the inoculated cells to allow viral growth to proceed.

In accordance with another aspect of the invention is a diagnostic kit for assaying a virus includes (A) cells from a cell line susceptible to infection by the virus; (B) a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX, or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and (C) reagents for assaying the virus.

In another aspect of the invention is a method for culturing a virus, comprising the steps of: (1) inoculating treated cells from a cell line susceptible to infection by the virus with the specimen, wherein the cells are treated with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a

-5-

cation; and (2) incubating the inoculated cells to allow viral growth to proceed.

In accordance with still another aspect of the invention is a method for assaying a virus, includes (A) providing cells from a cell line susceptible to infection by the virus and a specimen; (B) treating the cells with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX, or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; (C) inoculating the treated cells with the specimen; (D) incubating the inoculated cells to allow viral growth to proceed; and (E) assaying the virus in the cells.

In accordance with a further aspect of the invention is an enhanced mink lung cell line having improved susceptibility to infection by a herpes virus prepared by treating mink lung cells identified as ATCC #CCL64 with a compound of the formula $RG(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation.

In another aspect of the invention, the method relates to a diagnostic kit for assaying a virus, comprising: (1) treated cells from a cell line susceptible to infection by the virus, wherein the cells have been treated with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and (2) reagents for assaying the virus.

In another aspect of the invention, the method relates a method for assaying a virus, comprising the steps of: (1) inoculating treated cells

-6-

from a cell line susceptible to infection by the virus, wherein the cells have been treated with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and treated cells with the specimen; (2) incubating the inoculated cells to allow viral growth to proceed; and (3) assaying the virus in the cells.

The claimed methods, diagnostic kits and enhanced cell lines provide and have improved sensitivity for virus assay and enhanced susceptibility to virus infection.

Brief Description of the Drawings

Figure 1 (a-c) are photographs of immunofluorescent stained nuclei of CMV grown on sodium butyrate treated mink lung cells, untreated mink lung cells and MRC-5 cells.

Figure 2 (a-b) are photographs of immunofluorescent stained HSV-1 infected cells grown in sodium butyrate treated and untreated mink lung cells.

Figure 3 (a-b) are photographs of immunofluorescent stained HSV-2 infected cells grown in sodium butyrate treated and untreated mink lung cells.

Description of the Preferred Embodiments

The term "hydrocarbyl" includes hydrocarbon as well as substantially hydrocarbon groups. Substantially hydrocarbon groups describes groups which contain heteroatoms substituents that do not alter the predominantly hydrocarbon nature of the substituent. Examples of hydrocarbyl groups include the following:

-7-

(1) hydrocarbon substituents, i.e., aliphatic (e.g., alkyl or alkenyl) and alicyclic (e.g., cycloalkyl, cycloalkenyl) substituents, aromatic-, aliphatic- and alicyclic-substituted aromatic substituents and the like, as well as cyclic substituents wherein the ring is completed through another portion of the molecule (that is, for example, any two indicated substituents may together form an alicyclic radical);

(2) substituted hydrocarbon substituents, i.e., those substituents containing non-hydrocarbon groups which, in the context of this invention, do not alter the predominantly hydrocarbon nature of the substituent; those skilled in the art will be aware of such groups (e.g., halo, hydroxy, mercapto, nitro, nitroso, sulfoxy, etc.);

(3) heteroatom substituents, i.e., substituents which will, while having a predominantly hydrocarbon character within the context of this invention, contain an atom other than carbon present in a ring or chain otherwise composed of carbon atoms (e.g., alkoxy or alkylthio). Suitable heteroatoms will be apparent to those of ordinary skill in the art and include, for example, sulfur, oxygen, nitrogen and such substituents as, e.g., pyridyl, furyl, thienyl, imidazolyl, etc.

In general, no more than about 2, preferably no more than 1 heteroatom substituent will be present for every 10 carbon atoms in the hydrocarbyl group. Typically, there will be no such heteroatom substituents in the hydrocarbyl group. Accordingly, the hydrocarbyl group is purely hydrocarbon.

In this specification and in the appended claims the term "isomer" is to be given its standard meaning in the art and includes, but is not limited to, both structural and optical isomers as well as other types of isomers known in the art.

-8-

In this specification and in the appended claims a "herpes virus" is meant to describe the herpesviridae viruses which include but are not limited to cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes virus 6, Varicella-Zoster virus (VZV) and herpes simplex virus (HSV). "Chlamydiae" is meant to include chlamydia psittaci, chlamydia pneumoniae and chlamydia trachomatis. "Adenoviruses" are meant to include all types of human adenovirus as well adenoviruses which infect other organisms.

In this specification and the appended claims the term "cytopathic effect (cpe)" is defined as morphological changes which are induced in the cell as a result of viral infection of the cell. Such morphological changes may be syncytial, ballooning, enlargement, round-up, etc.

It is also to be understood that, in this specification and in the appended claims, assaying includes detecting and/or quantitating as well as other means of characterization of the virus known in the art.

It is also to be understood that although the invention is described in terms of viruses, the techniques and processes of the present invention may be used for culturing and/or assaying other types of micro-organisms which may be cultured in cells. An example of such a micro-organism is chlamydiae and for purposes of this specification and appended claims such micro-organisms which can be cultured in cells are meant to be included within the scope of the term "virus" although they are not actually viruses.

The method for culturing a virus of the present invention includes the steps of: (A) providing cells from a cell line susceptible to infection by the virus and a specimen; (B) treating the cells with a compound of formula $RC(O)Q$, wherein Q is R, OR, OX, or X, each R is independently

-9-

hydrogen or a hydrocarbonyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; (C) inoculating the treated cells with the specimen; and (D) incubating the inoculated cells to allow viral growth to proceed.

5 Generally, the present invention may be used to culture any virus which may infect a cell line in vitro. Specific examples include but are not limited to herpes viruses, the adenoviruses, paramyxoviridae viruses, influenza viruses, enteroviruses, and chlamydiae.

10 In one embodiment, the virus is herpes simplex virus (HSV), including HSV-1, HSV-2, cytomegalovirus and varicella zoster virus. HSV-1 and HSV-2 belong to the genus simplex virus of the herpes virus family. The specific properties of HSV-1 and HSV-2 are variable host range, relatively short growth cycle, rapid spread in culture, efficient destruction of infected cells, and the capacity to establish latency,
15 primarily in sensory ganglia.

 In one embodiment, the virus is cytomegalovirus (CMV). CMV is a herpes virus and has typical herpes virus morphology including a large DNA genome and the ability to establish persistent and latent infections. CMV is a member of the beta-herpes group which also includes herpes
20 virus 6 and herpes virus 7. CMV has distinguishing characteristics which include salivary gland tropism, species specificity, and slow growth in tissue culture.

 In one embodiment, the virus is an adenovirus. In another embodiment, the virus is a human adenovirus. Human adenoviruses are
25 classified in the adenovirus family and in the genus Mastadenovirus. Adenovirus is a DNA virus and based on DNA homology, the 47 human

-10-

adenovirus serotypes divide into 6 subgenera. Examples of adenoviruses include human adenovirus type 3 and adenovirus type 5.

5 In one embodiment, the viruses are in the family of paramyxoviridae. These viruses include genus of paramyxovirus, pneumovirus and mobilivirus. The important human pathogens of this group of viruses are respiratory syncytial virus, measles virus, parainfluenza virus type 1, 2, 3, 4, and mumps virus. The genome of this group of virus is nonsegmented, negative sense RNA. This group of viruses are transmitted via respiratory secretions through close person to person contact and aerosols. The virus initiates infection by invasion of the respiratory epithelial cells. The clinical manifestations include croup, pneumonia, bronchiolitis and common cold, and measles for measles virus and parotitis and orchitis for mumps virus. Respiratory syncytial virus is the most common lower respiratory tract infection of infants and young children. The second common one is parainfluenza virus type 3.

10 In one embodiment, the virus is an influenza virus. Influenza viruses belong to the family of Orthomyxoviridae, which contains influenza virus type A, influenza virus type B and influenza virus type C. The genome is segmented, single-stranded and negative sense RNA. The virus transmitted by infected respiratory secretions and replicate in the respiratory epithelial cells. The common clinical syndromes of Influenza A or B infections are fever, myalgias and cough, with clinical syndromes of common cold, pharyngitis, croup, bronchiolitis and pneumonia. Influenza A causes about 2 epidemics every 3 years and Influenza B is once every 3 years. Influenza C associates with subclinical or common cold like illness and does not cause recognized epidemics.

- 11 -

In one embodiment, the virus is an enterovirus. Enterovirus and rhinovirus are the two genera of the family of picornaviridae. Enteroviruses have been subdivided into polioviruses type 1-3, coxsackieviruses group A type 1-24, group B type 1-6, echoviruses type 1-34 and enteroviruses type 68-72. The virus contains a linear, single-stranded RNA genome. The RNA is infectious. Initially, ingested virus implants and replicates in susceptible tissues of the pharynx or distal part of the gut. The majority of enterovirus infections are asymptomatic, but in some cases, a variety of different clinical syndromes may occur such as respiratory illnesses, aseptic meningitis, hand-foot-and-mouth disease, herpangina, acute hemorrhagic conjunctivitis, paralysis etc.

In one embodiment, the virus is chlamydiae. The chlamydiae are actually not a virus, but rather non-motile, gram-negative, obligate intracellular bacteria which may be cultured in cells. There are three species of chlamydiae: chlamydia trachomatis, chlamydia psittaci and chlamydia pneumoniae.

The cells provided are from a cell line generally characterized as being susceptible to infection by the virus to be cultured or assayed. Examples of cell lines useful in the present invention include but are not limited to mink lung cells, McCoy cells, buffalo green monkey kidney cells, human lung carcinoma A549 cells, African green monkey kidney cell line, Rhesus monkey kidney cell line (LLC mk-2), Baby hamster kidney cell line (BHK), and Human lung mucoepidermoid carcinoma cell line (NCI-H292). In one embodiment, the cell lines are nonhuman cell lines.

Mink Lung cells are available from the American Type Culture Collection (ATCC), Rockville, Md as mink lung cell line ATCC No. CCL64.

-12-

Mink lung cells are obtained from the trypsinized lungs of near term, unsexed fetuses of Aleutian minks. McCoy cells are of mouse origin and are available from ATCC as ATCC No. CRL 1696. A549 cells are lung carcinoma cells of human origin and are available from ATCC as ATCC No. CCL185. Buffalo green monkey kidney cells (BGMK) are available from BioWhittaker Co. of Walkersville, Md. African green monkey kidney cell lines are available from ATCC as the following cell lines and their corresponding catalog numbers: CV-1, CCL-70; BSC-1, CCL26; VERO, CCL 81; VERO 76, CRL 1587, VERO C-1008, CRL 1586. Rhesus monkey kidney cell line LLC mk-2 is available from ATCC as ATCC CCL-7. Baby hamster kidney cell lines are available from the ATCC as following cell lines and their corresponding ATCC catalog numbers: BHK 21, CCL10; and BHK 21C13, CRL 8544. Human lung mucoepidermoid carcinoma cell line NCI-H292 is available from ATCC as ATCC No. CRL 1848. Although the subject invention is discussed in terms of established cell lines, it is also contemplated that the methods or compositions of the invention may find use with primary cell cultures as well as non-established or finite cell lines.

In one embodiment, the cell line may be a genetically engineered cell line. In one embodiment, the cell line is selected from the group consisting of McCoy cells and BGMK cells and the virus is a chlamydiae.

The cell lines and the viruses may be used in any combination. In one embodiment, the cell line is human lung carcinoma A549 cells and the virus is an adenovirus. A549 cells are generally used to culture adenovirus because of its susceptibility to adenovirus infection. In a further embodiment, the cell line is a mink lung cell line and the virus is

-13-

a herpes virus. The following chart provides further embodiments of the combinations of cell lines and viruses.

	<u>Virus</u>	<u>Cell Line</u>
5	1. Paramyxoviridae	African green monkey kidney cell lines Rhesus monkey kidney cell line (LLc mk-2) Mink lung cell line
	2. Influenza viruses	African green monkey kidney cell lines Rhesus monkey kidney cell line (LLc mk-2) Mink lung cell line
10	3. Adenoviruses	African green monkey kidney cell lines
	4. Enteroviruses	African green monkey kidney cell lines Rhesus monkey kidney cell line LLc mk-2) Human lung mucoepidermoid carcinoma cell line (NCI-H292)
15	5. Human herpes viruses	African green monkey kidney cell lines Baby hamster kidney cell line_(BHK) Mink lung cell line.

As recited above, a specimen is provided. The specimen provided may or may not be infected with or contain the virus. If the culturing method is to be used to provide virus for preparation of vaccine or viral proteins, etc., the specimen is pure virus or a specimen known to contain a virus.

Examples of suitable specimens for use in the present invention include body fluids, swabs, aspirates and washes from body cavities, body lesions, and various body tissues suspected of being infected with the virus or microorganism, as well as aliquots of solutions containing the virus or pure virus.

-14-

In one embodiment, the specimen is suspected of CMV infection. Examples of suitable specimens include urine, blood, eye swabs, throat swab, cerebral spinal fluid (CSF), rectal swab, bronchial wash, lung tissue, liver tissue, kidney tissue, heart tissue and brain tissue. In
5 another embodiment, the specimen is suspected of herpes simplex virus infection. Examples of suitable specimens include vesical lesions, cervical swabs, mouth swabs, CSF, eye swabs, brain tissue, liver tissue and lung tissue. In still another embodiment, the specimen is suspected of adenovirus infection. Examples of suitable specimens include nasal
10 swabs or expectorant, throat swabs, stool, urine, eye swabs and washings, nasopharyngeal washings and CSF. In a still further embodiment, the specimen is suspected of a chlamydia infection. Examples of suitable specimens include conjunctiva from eye infections, anterior urethra or cervix from genital infection, nasopharyngeal or
15 tracheobronchial aspirates from pulmonary infection.

In one embodiment, the specimen is pure virus or other microorganism obtainable from various depositories such as the ATCC or commercial sources known in the art. In a further embodiment, the specimen is as described above and is known to contain a virus.

20 As described above the cell line provided is treated with a compound of formula $RC(O)Q$, wherein Q is R, OR, OX, or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation.

In one embodiment, R may be an alkyl, hydroxyalkyl, or haloalkyl
25 group containing 1 to about 10 carbon atoms, or an alkoxyalkyl group containing 2 to about 10 carbon atoms. In another embodiment, R may be an alkyl group containing 1 to about 6 carbon atoms.

-15-

Specific examples of alkyl groups containing from one to about 10 carbon atoms include methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, cyclopentyl, cyclohexyl, 2-ethylhexyl groups as well as isomers thereof. Specific examples of hydroxyalkyl groups containing one to about 10 carbon atoms include hydroxymethyl, hydroxyethyl, and the various isomers of hydroxypropyl, hydroxybutyl, hydroxypentyl, hydroxyhexyl, hydroxyheptyl, hydroxyoctyl, hydroxynonyl, and hydroxy-decyl groups. Specific examples of alkoxyalkyl groups containing two to about 10 carbon atoms include methoxymethyl, ethoxymethyl, butoxy-methyl, butoxybutyl, etc. Specific examples of haloalkyl groups containing one to about 10 carbon atoms include the various isomers of chloromethyl, bromomethyl, iodomethyl, chloroethyl, bromoethyl, bromopropyl, iodoethyl, chlorobutyl, bromobutyl, iodobutyl, chloropentyl, bromopentyl, etc.

Generally, X is hydrogen or a cation. In one embodiment, X is hydrogen. In another embodiment, X is a cation. In still another embodiment, X is an alkali metal cation. In a further embodiment, X is a sodium cation. Suitable examples include but are not limited to lithium, sodium, potassium, cesium and ammonium cations.

In one embodiment, the compound of formula $RC(O)Q$ is butyric acid and salts thereof. In another embodiment, the compound of formula $RC(O)Q$ is sodium butyrate.

Generally, the cell line is treated with an amount of the compound of formula $RC(O)Q$ which is sufficient to enhance the cell line susceptibility to infection by the virus. In general, the amount of the compound used to treat the cell line will vary with the cell line and virus being used. Generally, the cell lines may be treated at a concentration of

-16-

about 0.1 mM up to the viability limit of the cell line. The term viability limit refers to the concentration level of the compound which is toxic to the cell line and the point at which the cell line is no longer viable for infection. The cell line would preferably be viable for at least about 12 hours, preferably from about 1 to about 30 days, more preferable from about 3 to about 15 days after treatment with the compound. In one embodiment, the cell line is treated with the above described compound at a concentration from about 0.1 mM to about 250 mM, or from about 2 to about 240 mM, or from about 5mM to about 180mM. In one embodiment, the cell line is treated with the compound, such as sodium butyrate, having a concentration from about 0.1mM to about 250mM, or from about 0.1mM to about 200mM or from about 1mM to about 175mM, or from about 2.5mM to about 150mM. It is to be understood that, in this specification and in the appended claims, the range and ratio limits recited herein, may be combined.

In one embodiment, the cell line is a mink lung cell line and the virus is CMV and the mink lung cell line is treated with the compound, such as sodium butyrate, having a concentration of about 40mM to about 120mM, or about 50mM to about 110mM, or about 60mM to about 100mM. In another embodiment, the cell line is a mink lung cell line and the virus is a herpes simplex virus and the mink lung cell line is treated with sodium butyrate having a concentration of about 0.1mM to about 20mM, or about 0.5mM to about 18mM, or about 1mM to about 15mM.

In one embodiment, the cell lines are those infected by the paramyxoviridae and influenza viruses, such as African green monkey kidney cell line, Rhesus monkey kidney cell line, Mink lung cell line, and

-17-

Mink lung cell lines. These cell lines are treated with the compound at a concentration from about 10mM to about 180mM, or from about 15 mM to about 120mM, or from about 20mM to about 80mM.

5 In another embodiment, the cell lines are those infected by the adenovirus and enterovirus, such as African green monkey kidney cell line, Rhesus monkey kidney cell line, and Human lung mucoepidermoid carcinoma cell line. These cell lines are treated with the compound at a concentration from about 0.1mM to about 80mM, or from about 1 mM to about 50mM, or from about 2mM to about 30mM, or from about
10 5mM to about 10mM.

Generally, the sodium butyrate is prepared in a solution of tissue culture media by weighing out the appropriate amount of sodium butyrate according to the desired concentration and dissolving it in the tissue culture medium. However, it is to be understood that the compound of
15 formula RC(O)Q may be prepared in a solution of any solvent or solution which will not inhibit or otherwise negatively impact upon viral growth. For example, the sodium butyrate may be prepared in water.

The tissue culture media used is generally any media which will support cell culture. For instance, the tissue culture medium may be
20 Opti-MEM which is available from Life Technologies, Inc. of Gaithersburg, Maryland. Opti-MEM is a modification of Minimum Essential Medium (MEM), buffered with 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) and sodium bicarbonate and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements and growth
25 factors. MEM generally contains inorganic salts, D-glucose, phenol red sodium succinate, succinic acid, amino acids and vitamins.

-18-

The cells of the cell line are generally treated with the compound of Formula I for a time sufficient to allow enhancement of cell line susceptibility to viral infection. In one embodiment, the cells are treated from about 1 hour to about 72 hours, or from about 8 hours to about 56 hours, or from about 12 hours to about 36 hours, with the compound of formula RC(O)Q.

Once treated the cells are inoculated with a specimen, described above, which is suspected of being or known to be infected by the virus or the cell line may be inoculated with the actual virus. Inoculation is accomplished by contacting the specimen with the treated cells and is done by any technique known in the art which will effectively contact the specimen with the cell line.

The specimen inoculum is prepared as follows. Clinical specimens are collected from an infected site such as the throat, nose, vesicle lesions, urine, etc., and put into a viral transport medium. Any viral transport media known in the art may be used. Generally, such media contain a buffer, protein and various antibiotics and/or fungicides such as penicillin, streptomycin, amphotericin, etc. The transport media mixture is generally vortexed and filtered through a membrane to eliminate contamination. At this point the inoculum is ready to be inoculated into the cell culture.

After inoculation, the inoculated cells are incubated to allow viral growth or infection to proceed. In one embodiment, the inoculated cells are incubated from about 1 hour to about 72 hours, or from about 8 hours to about 56 hours, or from about 12 hours to about 36 hours at a temperature conducive to viral growth. In one embodiment, the inoculated cells are incubated from about 25°C to about 50°C, or about

-19-

30°C to about 45°C, or from about 35°C to about 40°C. In one embodiment, the inoculated cells are incubated at about 37°C.

In another embodiment, the culturing method may include a further step (E) harvesting said virus. The harvested virus can be used in the production of vaccines, viral proteins, viral antigens, antibodies, etc. The viruses are harvested according to methods known in the art depending on the specific character of the virus and the cells in which they are grown. For viruses which are released into the tissue culture medium, the viruses are collected at the peak of cytopathic effect. Generally, the virus containing medium is collected. If cells or cell remnants remain in the medium, the medium may be centrifuged to pellet the cell material for removal. For viruses which are cell associated and do not release efficiently into the tissue culture medium, the cell as well as the medium needs to be harvested. The cell is then sonicated or quick-frozen and thawed to release the virus from the cells. The cell debris is then pelleted and removed by centrifugation. Subsequently, most viruses are generally stable when stored at about -70°C.

In another aspect of the invention a method for assaying a virus in a specimen is contemplated. The method includes the steps of (A) providing cells from a cell line susceptible to infection by the virus and a specimen; (B) treating the cell line with a compound of formula $RC(O)Q$, wherein Q is R, OR, OX, or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; (C) inoculating the treated cells with the specimen; (D) incubating the inoculated cells to allow viral growth to proceed; and (E) assaying the virus grown in the cells.

-20-

Steps (A), (B), (C) and (D) as well as the compositions and components used therein are as described above. In step (E) the virus is assayed in the specimen using any suitable method known in the art. Examples of such methods include tube culture, shell vial, PCR, Elisa and direct antigen staining.

In one embodiment, the virus is assayed by immunofluorescent staining. Generally, immunofluorescent staining involves staining the infected cells with an antibody or antibodies directed to a particular antigen produced by the virus. A fluorescent compound is attached to the antibody or to a related compound so that when the antigen-antibody complex is formed viral presence is visualized. The fluorescence tagged antibody-antigen complex is viewed using a fluorescence equipped microscope.

The following examples illustrate the processes and compositions of the present invention. Unless otherwise indicated in the following examples and elsewhere in the specification and claims, all parts and percentages are by weight, all temperatures are in degrees Centigrade, and pressure is at or near atmospheric pressure.

Example 1

Mink lung cells available from American Type Culture Collection ATCC No. CCL64 are provided. The cells are received in frozen vials from ATCC and are prepared by first thawing in a 37°C water bath. The thawed cells are removed from the bath and cultured in a tissue culture flask with Opti MEM culture medium supplemented with 5% fetal calf serum and non-essential amino acids, available from Life Technologies, Inc., of Gaithersburg, Maryland, and the antibiotics penicillin (100 units/ml), streptomycin (100 µg/ml) and the fungizone amphotericin (0.25

-21-

$\mu\text{g/ml}$). The cultured cells reach a complete monolayer in about 3 to 4 days. The mink lung cells are propagated by trypsinization and approximately 2.5×10^5 dispersed cells per shell vial are seeded in shell vials with cover slips. The shell vials are incubated at 37°C for 24 hours or until the cells form a monolayer.

5 The cells were then treated with a 5mM sodium butyrate (NaB) tissue culture solution, prepared by dissolving the appropriate amount of NaB in tissue culture media and filtering the solution, and incubated for 16 to 24 hours. After incubation the NaB and tissue culture media is removed and the treated cells are inoculated with 0.2ml of CMV
10 inoculum originally from a clinical specimen obtained from urine and prepared by putting the specimen into a viral transport medium containing Hank's buffer, 1% bovine albumin and the antibiotics penicillin (200 units/ml) and streptomycin ($200 \mu\text{g/ml}$) and the fungizone amphotericin
15 ($0.5 \mu\text{g/ml}$). After inoculation the shell vials are centrifuged at 700xg for 45 minutes at 23°C . After centrifugation, 0.5ml of fresh media is added to each vial and the vials are incubated at 37°C for 16 to 24 hours. After incubation the media is removed and the cell layers are rinsed with phosphate buffered saline (PBS), fixed with cold acetone for 10 minutes, and rinsed again with PBS.
20

After fixing, the cells are stained by adding $100 \mu\text{l}$ of CMV monoclonal antibody (mAb), incubated at 37°C for 45 minutes and then rinsed with PBS. The CMV mAb used is E-13 monoclonal antibody against immediate early (IE) antigen of CMV. This antibody is used as a
25 primary antibody in the first step of staining and is available from Accurated Chemical and Scientific Co., Westbury, N.Y. After the first staining step $100 \mu\text{l}$ of biotin which is conjugated with antimouse IgG is

-22-

added followed by additional incubation at 37°C for 45 minutes. The biotin complex is available from Boehringer Mannheim Co., Indianapolis, IN. The cells are rinsed again with PBS after incubation and then incubated with 100 μ l of streptavidin conjugated with fluorescein isothiocyanate (FITC) for 45 minutes at 37°C. The streptavidin complex is also available from Boehringer Mannheim Co., Indianapolis, IN. After a final rinse with PBS, a coverslip removed from the shell vial is mounted on the slide which is then examined under a fluorescence equipped microscope manufactured in Germany by Carl Zeiss, Inc., and available from W. Nuhsbaum, McHenry, IL. The specific apple-green stained nuclei were counted. The results are reported in Table 1 as positive stained nuclei as an average of four shell vials.

Examples 2-6

These examples used the same procedure as Example 1, except that the concentration of NaB used was different. Table 1 indicates the concentration of NaB used and the results.

Conventionally, human embryonic lung (HEL) fibroblast cells, such as the MRC-5 cell line which is available from Diagnostic Hybrids, Inc., Athens, OH, are used worldwide for CMV culture and viral isolation.

Comparative Example 1

This example used the same procedure as Example 1 except the CMV was grown in MRC-5 cells. The MRC-5 cells were not treated with NaB before infection, but were treated with 2mM calcium chloride (CaCl_2), 1% dimethyl sulfoxide (DMSO) and 10^{-5} M dexamethasone after infection. The results are reported in Table 1.

-23-

TABLE 1

Ex.	NaB (mM)	# positive stained nuclei
1	5	22
2	10	30
3	20	38
4	40	71
5	80	85
6	0	8
COMP EX 1	MRC-5 cells	9

As seen from Table 1, untreated mink lung cells, are slightly less sensitive than MRC-5 cells for CMV isolation and detection. However, when treated with NaB, mink lung cells show far superior sensitivity for CMV isolation and detection. The number of stained cells increases as the concentration of NaB is increased up to 80mM with the increase being about 10 fold as compared with untreated mink lung cells.

Figures 1(a-c) depict (a) stained nuclei from CMV grown in NaB treated mink lung cells; (b) one stained nuclei (arrow, other apparent fluorescence is artifact) from CMV grown in untreated mink lung cells; and (c) one stained nuclei (arrow) from CMV grown in MRC-5 cells which were prepared according to the procedure of Example 1. The improvement in the NaB treated mink lung cells is apparent.

Examples 7-11

Mink lung cells were prepared as described in Example 1. The mink lung cells were treated with various concentrations of sodium

-24-

butyrate for 16 to 24 hours as indicated in Table 1. Cells were then infected with HSV-1 (originally from clinical specimens but passaged once in tissue culture so virus content is assured). The HSV-1 culture fluid was diluted 1:1000 before use for inoculation. In each shell vial, 0.2 ml of inoculum was used. After centrifuging for 45 minutes at 700 xg at 23°C, 0.5 ml of Opti-MEM with 2% fetal calf serum was added to each vial and incubated overnight at 37°C. The following day, the cytopathic effect of the inoculated culture was observed under light microscope. The results are reported in Table 2 and are from the average of two shell vials.

Cell culture isolation and detection of HSV-1 from clinical specimens is conventionally done using primary rabbit kidney (RK) cells. RK cells are available from Biowhittaker Co., Walkersville, MD. However, RK cells are primary cells so that animals need to be sacrificed each time cells are prepared. Consequently, the procedure for obtaining the cells is quite tedious and costly.

Comparative Example 2

This example used the same procedure as Example 7 except that rabbit kidney cells were used to grow the virus and the RK cells were not treated with NaB. The results are reported in Table 2.

-25-

TABLE 2

Ex.	NaB (mM)	cpe ¹
7	2.5	18
8	5.0	20
9	10.0	22
10	20	12
11	0	6
Comp Ex 2	RK cells	26

¹ cpe = foci of cytopathic effect

As seen from Table 2, untreated mink lung cells, are less sensitive than RK cells for HSV-1 isolation and detection. However, when treated with NaB, mink lung cells show comparable sensitivity for HSV isolation and detection. Also, Table 2 shows that in comparison to growth of CMV in mink lung cells wherein the optimum concentration appears to be about 80mM, for HSV-1 growth optimum concentration is shown to be about 5mM to about 10mM.

Examples 12-21

These examples were prepared using the same procedure as Example 1, except that the concentration of NaB used was varied and both mink lung cells and MRC-5 cells were used. Also, the MRC-5 were treated with NaB before infection and 2mM CaCl₂, 1% DMSO and 10⁻⁵M dexamethasone after infection. Table 3 indicates the concentration of NaB used and the results obtained.

-26-

TABLE 3

Ex.	NaB- (mM)	Mink lung cells # of stained nuclei	MRC-5 cells # of stained nuclei
12	0	1	1
13	0	6	2
14	10	7	2
15	10	9	3
16	20	12	2
17	20	12	2
18	40	12	4
19	40	23	9
20	80	26	7
21	80	32	6

As seen from Table 3, mink lung cells show a better response to NaB treatment, resulting in a greater sensitivity to CMV infection, than the conventionally utilized, NaB treated, MRC-5 cells.

Examples 22-27

These examples used the same procedure as Example 1 for CMV growth except that the age of the cells were varied, the cells were treated with 80mM sodium butyrate 24 hours before infection, and the MRC-5 cells were treated 2mM calcium chloride (CaCl_2), 1% dimethyl sulfoxide (DMSO) and 10^{-5}M dexamethasone after infection. Also, before infection the inoculum was diluted with Opti-MEM as indicated in Table 4. The results are reported in Table 4.

-27-

TABLE 4

Ex.	Cell Type	Cell Age	10 ⁰	10 ⁻¹	10 ⁻²
22	Mink Lung *	2 days	232/220	44/52	5/2
23	Mink Lung	2 days	28/48	4/2	1/0
24	Mink Lung *	3 days	242/309	47/43	1/2
25	Mink Lung	3 days	28/29	4/2	2/0
26	MRC-5 *	2 days	39/29	3/1	0/0
27	MRC-5	2 days	20/22	1/1	1/0

In Table 4 * indicates cells treated with sodium butyrate, 10⁰ indicates no dilution, 10⁻¹ indicates 1:10 dilution, 10⁻² indicates 1:100 dilution and the two numbers indicate counts of positive stained nuclei from 2 shell vials. The Examples of Table 4 show the effects of cell age as well as virus concentration on viral growth. It is clear from Table 4 that even at a high dilution, i.e., low concentration of virus in the inoculum, the virus is still detected when the cells are treated with NaB. In contrast, untreated mink lung cells and both treated and untreated MRC-5 cells at times show no virus detected at high dilution.

Examples 28-32

These examples used the same procedure as Example 1 for CMV growth except that the age of the cells were varied, the cells were treated with 80mM sodium butyrate 24 hours before infection, and the MRC-5 cells were treated 2mM calcium chloride (CaCl₂), 1% dimethyl sulfoxide (DMSO), and 10⁻⁵M dexamethasone after infection. The results are reported in Table 5. The cell age is measured as the time after seeding of the cells in the shell vial.

-28-

TABLE 5

Ex.	Cell Type	Cell Age	Treated	Untreated
28	Mink Lung	2 days	123 *	11
29	Mink Lung	3 days	121	10
30	Mink Lung	5 days	78	4
31	Mink Lung	7 days	30	2
32	MRC-5	4 days	4	3

* average # of nuclei staining from three shell vials

As shown in Table 5, 2 and 3 day old mink lung cells show greater sensitivity to CMV growth than 5 to 7 day old mink lung cells. Furthermore, NaB treatment enhanced the susceptibility of the mink lung cells to CMV infection despite the age of the mink lung cells.

Examples 33-39

These examples used the same procedure as Example 1 for CMV growth except that the time of treatment with sodium butyrate was varied, i.e., the cells were treated with 80mM sodium butyrate for different times before infection, and the MRC-5 cells were treated with 2mM calcium chloride (CaCl_2), 1% dimethyl sulfoxide (DMSO) and 10^{-6}M dexamethasone after infection. The results are reported in Table 6.

-29-

TABLE 6

Ex.	Cell Type	Time of Treatment	# of Foci
33	Mink Lung	18 hours	360*
34	Mink Lung	8 hours	306
35	Mink Lung	6 hours	276
36	Mink Lung	4 hours	234
37	Mink Lung	2 hours	126
38	Mink Lung	Untreated	48
39	MRC-5	Untreated	30

* average # of stained nuclei counted from three shell vials.

The results of Table 6 indicate that as short as a 2 hour treatment of mink lung cells with NaB, more than a two fold enhancement of CMV expression can be detected. However, for optimum enhancement at least 8 hours of treatment is required.

Figures 2(a-b) and 3(a-b) depict 2(a) stained virus infected cells (appears yellow in the Figure) from HSV-1 grown in NaB treated mink lung cells; 2(b) stained virus infected cells from HSV-1 grown in untreated mink lung cells; 3(a) stained virus infected cells from HSV-2 grown in NaB treated mink lung cells; and 3(b) stained virus infected cells of HSV-2 grown in untreated mink lung cells. Figures 2 and 3 show that a much greater number of cells are infected in treated than in untreated cells. The virus were grown according to the procedures of Examples 7 and 11 with the exception that the virus infected cells were stained in a one step procedure with fluorescein isothiocyanate FITC conjugated mAB

-30-

against HSV-1 or HSV-2. This antibody is obtainable from Syva Co. of San Jose, CA.

5 In another aspect of the invention a diagnostic kit for assaying a virus, includes (A) cells from a cell line susceptible to infection by the virus; (B) a compound of formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and (C) reagents for assaying the virus is contemplated.

10 The cell line, virus and compound of formula $RC(O)Q$ are as described above. The reagents for assaying the virus can be any reagents known in the art which are used to assay viruses. Examples include but are not limited to immunological, colorimetric, fluorescent, optical, etc. In one embodiment, the reagents are immunofluorescent reagents such as those described above. Examples include but are not
15 limited to FITC-mAb to HSV-1 or HSV-2 (described above) and the E-13 mAb against immediate early antigen of CMV/biotin conjugated anti-mouse IgG/streptavidin-FITC conjugate system described above.

20 In still another aspect of the invention an enhanced mink lung cell line having improved susceptibility to infection by a herpes virus prepared by treating mink lung cells identified as ATCC #CCL64 with a compound of formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; is contemplated.

25 The mink lung cells and compound of formula $RC(O)Q$ are as described above. The enhanced mink lung cells are prepared by obtaining mink lung cells ATCC No. CCL64 from the ATCC. The cells are received in frozen vials from ATCC and are prepared by first thawing in a 37°C

-31-

water bath. The thawed cells are removed from the bath and cultured in a tissue culture flask with Opti MEM culture medium supplemented with 5% fetal calf serum and non-essential amino acids, available from Life Technologies, Inc., of Gaithersburg, Maryland, and the antibiotics penicillin (100 units/ml), streptomycin (100 μ g/ml) and the fungizone amphotericin (0.25 μ g/ml). The cultured cells reach a complete monolayer in about 3 to 4 days. The mink lung cells are propagated by trypsinization and approximately 2.5×10^5 dispersed cells per shell vial are seeded in shell vials with cover slips. The shell vials are incubated at 37°C for 24 hours or until the cells form a monolayer.

The cells are then treated with an appropriate concentration of sodium butyrate (NaB) prepared by dissolving the appropriate amount of NaB in tissue culture media and filtering the solution, and incubated for 16 to 24 hours.

Although the invention is described herein as being directed towards use in diagnosing viral presence, it is contemplated that any process or method, compound, composition, etc., which requires growth or culturing of viruses may find the present invention useful. For instance, the production of viral vaccines or viral proteins which may require culturing of the virus.

While the invention has been explained in relation to its preferred embodiments, it is to be understood that various modifications thereof will become apparent to those skilled in the art upon reading the specification. Therefore, it is to be understood that the invention disclosed herein is intended to cover such modifications as fall within the scope of the appended claims.

-32-

Claims

1. A method for culturing a virus, comprising the steps of:
 - (A) providing cells from a cell line susceptible to infection by the virus and a specimen;
 - 5 (B) treating the cells with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation;
 - (C) inoculating the treated cells with the specimen; and
 - 10 (D) incubating the inoculated cells to allow viral growth to proceed.
2. The method of claim 1, further comprising a step (E) harvesting said virus.
3. The method of claim 1, wherein R is an alkyl, hydroxyalkyl,
15 alkoxyalkyl, or haloalkyl group.
4. The method of claim 1, wherein Q is OX and X is hydrogen or an alkali metal cation.
5. The method of claim 3, wherein Q is OX and X is hydrogen or an alkali metal cation.
- 20 6. The method of claim 1, wherein the compound of the formula $RC(O)Q$ is butyric acid or salts thereof.
7. The method of claim 1, wherein the cell line is a genetically engineered cell line.
8. The method of claim 1, wherein the cell line is selected from
25 the group consisting of a mink lung cell line, McCoy cell line, buffalo green monkey kidney cell line, human lung carcinoma A549 cell line, African green monkey kidney cell lines, Rhesus monkey kidney cell line

-33-

(LLc mk-2), Baby hamster kidney cell line (BHK), and Human lung mucoepidermoid carcinoma cell line (NCI-H292).

9. The method of claim 1, wherein the virus is selected from the group consisting of herpes viruses, the adenoviruses, paramyxoviridae, influenza viruses, enteroviruses, and chlamydiae.

10. The method of claim 1, wherein the cell line is a mink lung cell line and the virus is a herpes virus.

11. The method of claim 6, wherein the cell line is a mink lung cell line and the virus is a herpes virus.

12. The method of claim 1, wherein the cell line is selected from the group consisting of McCoy cell line and buffalo green monkey kidney cell line and the virus is chlamydiae.

13. The method of claim 1, wherein the cell line is human lung carcinoma A549 cell line and the virus is an adenovirus.

14. A diagnostic kit for assaying a virus, comprising:

(A) cells from a cell line susceptible to infection by the virus;

(B) a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and

(C) reagents for assaying the virus.

15. The kit of claim 14, wherein R is an alkyl, hydroxyalkyl, alkoxyalkyl, or haloalkyl group.

16. The kit of claim 14, wherein Q is OX and X is hydrogen or an alkali metal cation.

17. The kit of claim 15, wherein Q is OX and X is hydrogen or an alkali metal cation.

-34-

18. The kit of claim 14, wherein the compound of the formula $RC(O)Q$ is butyric acid or salts thereof.

19. The kit of claim 14, wherein the cell line is a genetically engineered cell line.

5 20. The kit of claim 14, wherein the cell line is selected from the group consisting of a mink lung cell line, McCoy cell line, buffalo green monkey kidney cell line, human lung carcinoma A549 cell line, African green monkey kidney cell line, Rhesus monkey kidney cell line, Baby hamster kidney cell line (BHK), and Human lung mucoepidermoid carcinoma cell line.

10 21. The kit of claim 14, wherein the virus is selected from the group consisting of herpes viruses, the adenoviruses, paramyxoviridae, influenza viruses, enteroviruses, and chlamydiae.

15 22. The kit of claim 14, wherein the cell line is a mink lung cell line and the virus is a herpes virus.

23. The kit of claim 18, wherein the cell line is a mink lung cell line and the virus is a herpes virus.

20 24. The kit of claim 14, wherein the cell line is selected from the group consisting of McCoy cell line and buffalo green monkey kidney cell line and the virus is chlamydiae.

25 25. The kit of claim 14, wherein the cell line is human lung carcinoma A549 cell line and the virus is an adenovirus.

26. A method for assaying a virus, comprising the steps of:

25 (A) providing cells from a cell line susceptible to infection by the virus and a specimen;

 (B) treating the cells with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a

-35-

hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation;

(C) inoculating the treated cells with the specimen;

(D) incubating the inoculated cells to allow viral growth to proceed; and

(E) assaying the virus in the cells.

27. The method of claim 26, wherein R is an alkyl, hydroxyalkyl, alkoxyalkyl, or haloalkyl group.

28. The method of claim 26, wherein Q is OX and X is hydrogen or an alkali metal cation.

29. The method of claim 27, wherein Q is OX and X is hydrogen or an alkali metal cation.

30. The method of claim 26, wherein the compound of formula RC(O)Q is butyric acid or salts thereof.

31. The method of claim 26, wherein the cell line is a genetically engineered cell line.

32. The method of claim 26, wherein the cell line is selected from the group consisting of a mink lung cell line, McCoy cell line, buffalo green monkey kidney cell line, and human lung carcinoma A549 cell line, African green monkey kidney cell line, Rhesus monkey kidney cell line, Baby hamster kidney cell line (BHK), an dHuman lung mucoepidermoid carcinoma cell line.

33. The method of claim 26, wherein the virus is selected from the group consisting of herpes viruses, the adenoviruses, paramyxoviridae, influenza viruses, enteroviruses, and chlamydiae.

34. The method of claim 26, wherein the cell line is a mink lung cell line and the virus is a herpes virus.

-36-

35. The method of claim 30, wherein the cell line is a mink lung cell line and the virus is a herpes virus.

36. The method of claim 26, wherein the cell line is selected from the group consisting of McCoy cell line and buffalo green monkey kidney cell line and the virus is chlamydiae.

37. The method of claim 26, wherein the cell line is human lung carcinoma A549 cell line and the virus is an adenovirus.

38. An enhanced mink lung cell line having improved susceptibility to infection by a herpes virus prepared by treating mink lung cells identified as ATCC #CCL64 with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation.

39. The mink lung cell line of claim 38, wherein the compound of formula $RC(O)Q$ is butyric acid or salts thereof.

40. A method for culturing a virus, comprising the steps of:

(1) inoculating treated cells from a cell line susceptible to infection by the virus with the specimen, wherein the cells are treated with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and

(2) incubating the inoculated cells to allow viral growth to proceed.

41. The method of claim 40, further comprising a step (3) harvesting said virus.

42. A diagnostic kit for assaying a virus, comprising:

-37-

(1) treated cells from a cell line susceptible to infection by the virus, wherein the cells have been treated with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and

5

(2) reagents for assaying the virus.

43. A method for assaying a virus, comprising the steps of:

(1) inoculating treated cells from a cell line susceptible to infection by the virus, wherein the cells have been treated with a compound of the formula $RC(O)Q$, wherein Q is R, OR, OX or X, each R is independently hydrogen or a hydrocarbyl group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation; and treated cells with the specimen;

10

(2) incubating the inoculated cells to allow viral growth to proceed; and

15

(3) assaying the virus in the cells.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20827

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12Q 1/70; G01N 33/53; C12N 7/00, 7/02, 5/00

US CL : 435/5, 7.2, 7.21, 235.1, 239, 240.2, 240.23

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 7.2, 7.21, 235.1, 239, 240.2, 240.23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, BIOSIS, MEDLINE, EMBASE, SCISEARCH

search terms: herpes, HSV, cytomegalovirus, CMV, butyric acid, butyrate, sodium butyrate, mink lung cell line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	ASH. R.J. Butyrate-Induced Reversal of Herpes Simplex Virus Restriction in Neuroblastoma Cells. Virology. 1986. Vol. 155. pages 584-592, especilly pages 585-586.	1-6, 9 ----- 7, 8, 10, 11-37, 39-43
X ---- Y	Roubal. J. Effect of N-Butyrate on Superinfection of Raji lymphoblastoid Cell Line by Epstein-Barr Virus. Acta. virol. 1985. Vol 29. pages 162-165, especially page 163.	1-6, 9 ----- 7, 8, 10, 11-37, 39-43

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 06 MARCH 1997	Date of mailing of the international search report 25 MAR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BRETT L. NELSON Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20827

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	HOWARD. C. Comparison of Embryonic Mink Lung (MvLu) Cell and Shell Vials (SV) and Tubes and MRC-5 Sell Vials and Tubes for the Isolation of Herpes Simplex Virus (HSV). Abstracts of the 89th annual meeting of the American society for microbiology. 1989. C-2.	38 ----- 7, 8, 19, 11-37, 39-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20827

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-5, 14-17, 26-29, 38, and 40-43 (in part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20827

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

The claims are drawn to include a chemical formula which reads on an unsearchable number of compounds. Therefore, because the claims are broadly drawn to encompass a very large number of Group/species combination a meaningful search cannot be done. The examiner will search a mink lung cell line and a method for culturing herpes virus in a mink lung cell line which employs treated the susceptible cells with sodium butyrate or claims 6, 8-11, and 39.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claims 1-6, 38, and 39, sharing the inventive concept of a method for culturing a virus, and a mink lung cell line.

Group II, claims 14-18, sharing the inventive concept of a diagnostic kit.

Group III, claims 26-30, sharing the inventive concept of an assay method.

Group IV, claims 40 and 41, sharing the inventive concept of a method for culturing a virus employing pretreated cells.

Group V, claim 42, sharing the inventive concept of a diagnostic kit employing pretreated cells.

Group VI, claim 43, sharing the inventive concept of an assay method employing pretreated cells.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Roubal, et al. (Acta. Virol. 1985) teach a method of culturing Epstein-Barr virus in cells that have been pretreated with sodium-butyrate (p. 163, para.s 2 and 3), therefore, claim 1 lacks a special technical feature. Additionally, Groups I-VI are drawn to different methods, and PCT rules 13.1 and 13.2 do not provide for multiple independent methods of using different compositions.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. The species are as follows:

species 1) mink lung, McCoy, buffalo green monkey kidney, human lung carcinoma A549, African green monkey kidney, Rhesus monkey kidney (LLc mk-2), Baby hamster (BHK), and Human lung mucoepithelial carcinoma (NCI-H292) cell lines.

species 2) herpes virus, adenovirus, paramyxovirus, influenza virus, enterovirus, and chlamydiae.

species 3) the chemical compound RC(o)Q, wherein Q is R, OR, or X and each R is independently hydrogen or a hydrocarbonol group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation.

The claims are deemed to correspond to the species listed above in the following manner:

species 1) claims 8, 10, 11, 20, 22, 23, 32, 34, 35, 38, and 39-mink lung; claims 8, 12, 20, 24, 32, and 36-McCoy; claims 8, 12, 20, 24, 32, and 36-buffalo green monkey kidney; claims 8, 13, 20, 25, 32, and 37-human lung carcinoma A549; claims 8, 20, and 32-African green monkey kidney; claims 8, 20, 32-Rhesus monkey kidney (LLc mk-2); claims 8, 20, and 32-Baby hamster (BHK); and claims 8, 20, and 32 Human lung mucoepithelial carcinoma (NCI-H292) cell lines.

species 2) claims 9-11, 21-23, and 33-35-herpes virus; claims 9, 13, 21, 25, 33, and 37-adenovirus; claims 9, 21, and 33-paramyxovirus; claims 9, 21, 33-influenza virus; claims 9, 21, 33-enterovirus; and claims 9, 12, 21, 24, 33, and 36-chlamydiae.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20827

species 3) claims 1-5, 14-17, 26-29, and 40-43 correspond to the chemical compound $RC(O)Q$, wherein Q is R, OR, or X and each R is independently hydrogen or a hydrocarbon group containing 1 to about 10 carbon atoms and wherein X is hydrogen or a cation.

The following claims are generic: none

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the cell lines, viruses, and chemical compounds all represent different products to be used in the different methods and kits.